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# Natural-Abundance $^{13}C$ Nuclear Magnetic Resonance Spectra of Terpenes and Carotenes*

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**Abstract.** Natural-abundance $^{13}C$ nuclear magnetic resonance spectra are reported for some simple terpenes and carotenes. The techniques involved in the assignment of the resonances to specific carbons are outlined. The potential of this nondegradative procedure for structural analysis is demonstrated for the investigation of carbon atoms in chemical and biochemical systems.

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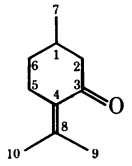
In a recent communication,[1] we demonstrated how the new technique of proton-noise decoupling has enabled us to obtain natural-abundance $^{13}C$ nuclear magnetic resonance spectra of several medium molecular weight organic compounds of biochemical interest. We now wish to show how the individual resonances in these spectra can be assigned to particular carbons by studies on closely related compounds and/or with the aid of specific[2] and off-resonance[3, 4] proton decoupling. The results for a group of terpenes and carotenoids reveal that, while $^{13}C$ chemical shifts are sensitive to even slight structural and stereochemical changes, the changes usually follow regular and predictable patterns. The ability so afforded to resolve and identify individual carbon atoms in complex substances provides an important nondegradative technique for the investigation of carbon atoms in chemical and biochemical systems.

The following general procedure has been used for the assignment of each of the spectra reported here. First, an initial and possibly tentative assignment of the resonances in the noise-decoupled spectrum is made from available model compounds and known substituent shifts.[4–9] An off-resonance proton-decoupled spectrum provides a check on the preliminary choice and separates the carbons according to their degree of substitution. If additional evidence is required, the specific proton-decoupling frequencies are determined.

Figure 1 shows the noise and off-resonance decoupled $^{13}C$ spectra of pulegone (I) which will be used to demonstrate the techniques involved. The number of hydrogens attached to each carbon atom is determined from the multiplicities of the peaks in the off-resonance decoupled spectrum. Off-resonance decoupled spectra provide the same information as undecoupled spectra but are more useful because the Overhauser effect associated with proton decoupling[10] is retained, and therefore the signal-to-noise ratio is improved. In addition, the magnitude of the apparent coupling constants within the multiplets can be manipulated by

changing the frequency offset or power of the decoupler and this can greatly aid the assignments where the lines overlap.




(I)

For (I), C3, C4, and C8 are identified by their chemical shifts which are characteristic of $\alpha,\beta$-unsaturated ketones. Further, C1 is the only carbon with a single hydrogen attached and is recognized as such by giving the only doublet in the off-resonance decoupled spectrum. The remaining six peaks are separated into two groups of three primary and three secondary carbon atoms according to the observed multiplicities of the proton couplings. The strong downfield shift induced by the carbonyl group on the adjacent carbon is used to assign C2. The choice between C5 and C6 can be made from exact proton-decoupling frequencies. The protons attached to C5 are known to absorb downfield of those at C6. When the exact proton-decoupling frequencies for the peaks at 159.6 and 164.0 ppm were determined, it was found that the 164 ppm peak was due to C5. Likewise, C7 can be differentiated from the other two methyl carbons, C9 and C10, by the same procedure. The problem of how to differentiate C9 and C10 still remains. Steric interactions are known to produce upfield shifts of carbon resonances[8, 11] and there is a small interaction between C9 and the carbonyl function, and so the upfield resonance is assigned to C9. This last assignment is tentative because of the small difference in shifts.

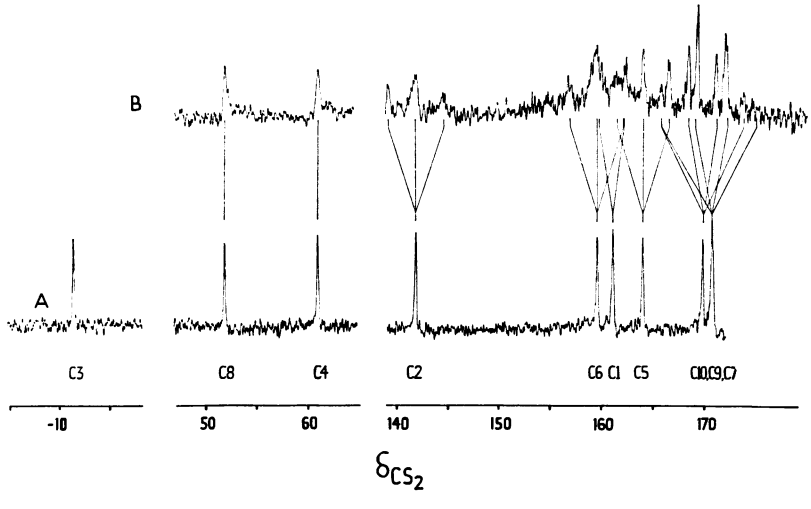
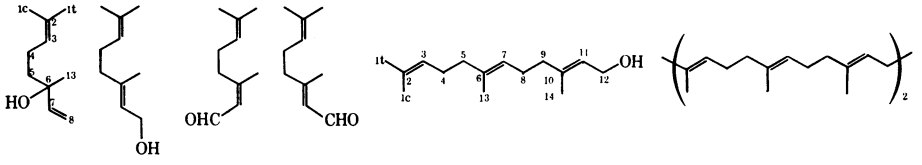



FIG. 1.—Natural abundance $^{13}C$ spectrum at 15.08 MHz of pulegone (I); A, 16 scans, noise decoupled; B, 80 scans, off-resonance decoupled.

Tables 1 and 2 show the cmr shifts for a number of other terpenes determined by similar procedures.

The carotene spectra of Table 3 illustrate how a correlation diagram for a series of closely related compounds can lead to an over-all assignment. The saturated carbon resonances for these substances are virtually identical and correspond well to the appropriate resonances in β-ionone. The assignment of the unsaturated carbon absorptions is more difficult because the range of shifts is smaller. Off-resonance decoupling allows separation of the peaks into those

TABLE 1. *$^{13}C$ chemical shifts of some acyclic terpenes.**

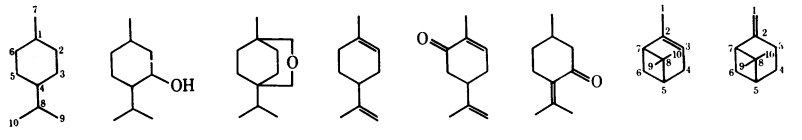



| Carbon | | Linalool | Geraniol | *cis*-Citral | *trans*-Citral | Farnesol | Squalene |
|---|---|---|---|---|---|---|---|
| *cis* | 1 | 175.0 | 175.1 | 175.1 | 175.1 | 175.1 | 175.0 |
| *trans* | 1 | 166.9 | 167.0 | 167.2 | 167.2 | 167.1 | 167.0 |
| | 2 | 61.8 | 61.3 | 59.6 | 60.2 | 61.7 | 61.9 |
| | 3 | 67.3 | 67.6† | 69.4 | 69.0 | 67.5† | 67.7† |
| | 4 | 164.7 | 165.9 | 165.3 | 166.5 | 165.6 | 165.5‡ |
| | 5 | 149.8 | 152.8 | 160.0 | 152.0 | 152.8‡ | 152.6 |
| | 6 | 119.9 | 55.2 | 30.4 | 30.4 | 57.6 | 57.7§ |
| | 7 | 46.5 | 68.0† | 63.8 | 65.0 | 68.2† | 67.9† |
| | 8 | 81.2 | 133.8 | 3.1 | 2.5 | 166.9 | 165.6‡ |
| | 9 | ... | ... | ... | ... | 152.7‡ | 152.6 |
| | 10 | ... | ... | ... | ... | 55.4 | 57.9§ |
| | 11 | ... | ... | ... | ... | 67.8† | 67.9† |
| | 12 | ... | ... | ... | ... | 133.9 | 164.1 |
| | 13 | 169.6 | 176.5 | 168.1 | 175.5 | 178.8§ | 176.6 |
| | 14 | ... | ... | ... | ... | 176.6§ | 176.6 |

* All shifts are in ppm upfield from $CS_2$.

†, ‡, and § represent groups of almost identical shifts where the assignment may possibly be reversed.

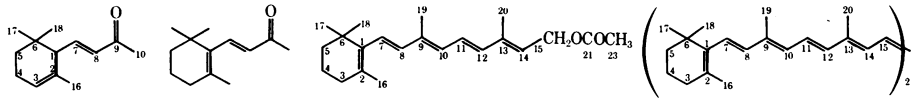
TABLE 2. *$^{13}C$ chemical shifts of some cyclic monoterpenes.**




| Carbon | Menthane | Menthol | Cineol | Limonene | Carvone | Pulegone | α-Pinene | β-Pinene |
|---|---|---|---|---|---|---|---|---|
| 1 | 156.8 | 160.6 | 123.4† | 59.3 | 57.3 | 161.1 | 171.7 | 86.2 |
| 2 | 159.4 | 146.9 | 160.8 | 71.7 | 48.7 | 141.8 | 48.4 | 41.2 |
| 3 | 162.6 | 121.6 | 169.5 | 161.9† | 161.2 | −8.6 | 76.3 | 168.7† |
| 4 | 148.4 | 142.1 | 159.4 | 151.3 | 149.8 | 60.8 | 161.0† | 168.9† |
| 5 | 162.6 | 169.0 | 169.5 | 164.5 | 149.4 | 164.0 | 151.0 | 151.7 |
| 6 | 159.4 | 157.5 | 160.8 | 161.6† | −5.2 | 159.6 | 161.1† | 165.5 |
| 7 | 170.0 | 170.2 | 165.1 | 168.7 | 177.1 | 170.8 | 145.2 | 140.4 |
| 8 | 156.8 | 166.7 | 119.5† | 42.8 | 45.3 | 51.7 | 154.5 | 151.7 |
| 9 | 173.5 | 176.5 | 163.7 | 84.1 | 82.2 | 170.7† | 166.0‡ | 166.7‡ |
| 10 | 173.5 | 171.5 | 163.7 | 172.0 | 172.3 | 169.8† | 169.7‡ | 170.8‡ |

* See footnotes to Table 1.

TABLE 3. *$^{13}C$ chemical shifts of the ionones and some carotenes.**




| Carbon | α-Ionone | β-Ionone | Vitamin A acetate | β-Carotene | 15,15′-*cis*-β-Carotene | 15,15′-Dehydro-β-carotene |
|---|---|---|---|---|---|---|
| 1 | 138.2 | 56.3† | 54.2† | 54.5† | 54.5† | 54.5† |
| 2 | 60.2 | 57.8† | 54.8† | 54.5† | 54.5† | 54.5† |
| 3 | 70.0 | 159.2 | 159.1 | 159.1 | 159.1 | 159.1 |
| 4 | 169.3 | 173.5 | 172.8 | 172.6 | 172.7 | 172.6 |
| 5 | 163.8 | 153.0 | 152.5 | 152.6 | 152.6 | 152.5 |
| 6 | 160.1 | 158.5 | 158.0 | 158.3 | 158.3 | 158.1 |
| 7 | 59.8 | 60.4 | 56.6 | 57.1 | 56.8 | 57.3 |
| 8 | 45.0 | 50.8 | 66.2 | 66.1 | 66.0 | 65.5 |
| 9 | −3.5 | −3.7 | 56.8† | 56.4† | 55.6† | 55.6† |
| 10 | 165.0 | 165.9 | 67.4‡ | 67.1‡ | 66.7‡ | 65.5‡ |
| 11 | ... | ... | 63.8 | 59.7 | 60.9 | 61.7 |
| 12 | ... | ... | 67.3‡ | 63.5‡ | 65.1‡ | 63.2‡ |
| 13 | ... | ... | 62.3 | 61.1 | 63.4 | 46.4 |
| 14 | ... | ... | 54.8 | 62.3‡ | 67.0‡ | 81.2 |
| 15 | ... | ... | 131.3 | 55.0 | 54.7 | 93.5 |
| 16 | 161.0 | 171.2 | 172.0 | 170.6 | 170.6 | 170.7 |
| 17 | 165.7 | 163.9 | 163.3 | 163.6 | 163.5 | 163.5 |
| 18 | 165.7 | 163.9 | 163.3 | 163.6 | 163.5 | 163.5 |
| 19 | ... | ... | 179.9 | 179.8 | 179.8 | 179.8 |
| 20 | ... | ... | 179.9 | 179.8 | 180.0 | 177.3 |
| 21 | ... | ... | 23.5 | ... | ... | ... |
| 22 | ... | ... | 170.7 | ... | ... | ... |

* See footnotes to Table 1.

which arise from tertiary and quaternary carbons. The C13, C14, and C15 resonances of the dehydrocarotene are readily identified by their chemical shifts. Because this acetylenic compound retains three quaternary resonances close to 55 ppm, these can be assigned to C1, C2, and C9, and the same carbon atoms can be identified in the remaining carotenes. The differentiation of C1, C2, and C9 has not been possible on any rational basis, although the fact that the highest field member of the group shows the greatest variation with structure suggests that this may be C9.

The correlation diagram for the unsaturated carbon resonances is shown in Figure 2, and was set up on the basis of the following criteria:

(*a*) The carbons farthest from the site of modification (i.e., C1, C2, C7, C8, C9) should only be slightly affected by changes at C15. This assumption has already been justified for C1, C2, and C9 and, hence, C7 and C8 can be extracted from the remainder.

(*b*) Carbons β to a methyl group appear upfield of those farther removed from a methyl group. This β-effect has been demonstrated for simple ethylenic compounds and is assumed to extend to conjugated systems. The resonances of C7, C11, and C15 fall into the low-field class and C7 has been differentiated from C8 on this basis. It is easy to confirm C15 because its resonance is missing in both vitamin A acetate and the dehydrocarotene. The fact that C11 is the most upfield member of this group is a consequence of steric interaction between

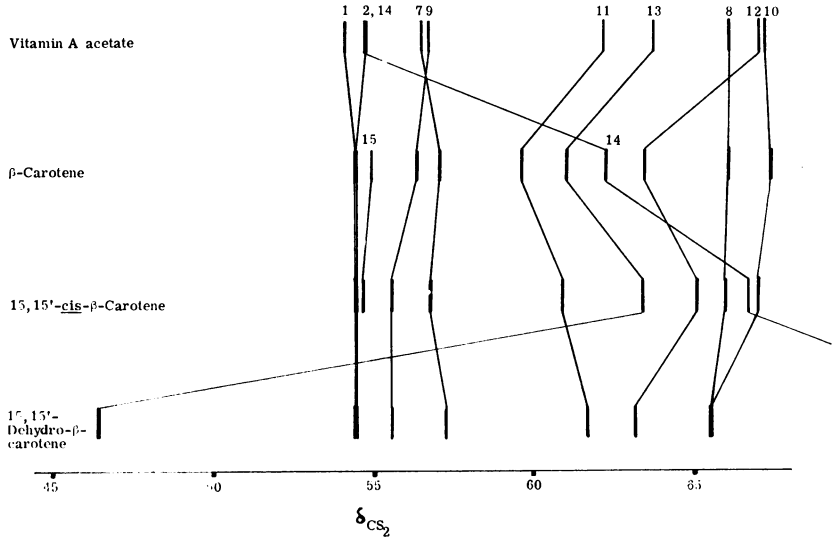



FIG. 2.—Correlation diagram for $^{13}C$ shifts of unsaturated carbon atoms in some carotenes.

the proton attached to this carbon and the C19 and C20 methyl groups (C7 and C15 have only one methyl interaction).

The C8, C10, C12, and C14 resonances are more difficult to differentiate. It seems reasonable to separate out C8 on the basis of its insensitivity to change at C15. The remaining assignments are quite tentative and are based on the assumption that the farther the carbon is from C15, the smaller will be the difference between isomers. This choice may have to be modified in the light of future evidence.

A similar study of the $^{13}C$ spectra of steroids has been completed and submitted for publication elsewhere.[12]

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